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Bureau voor de Industriële Eigendom

Hierbij wordt verklaard, dat in Nederland op 14 augustus 1998 onder nummer 1009871,

ten name van:

HOLLAND BIOMATERIALS GROUP B.V.

te Enschede

een aanvrage om octrooi werd ingediend voor:

"Inrichting voor het onderzoeken van chemische interacties en werkwijze die gebruik maakt van een dergelijke inrichting",

en dat de hieraan gehechte stukken overeenstemmen met de oorspronkelijk ingediende stukken.

Rijswijk, 31 augustus 1999.

De Directeur van het Bureau voor de Industriële Eigendom,
voor deze,

A.W. van der Kruk.

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

ABSTRACT

The invention relates to a device for investigating reactions between interactive species, said device comprising:

- one or more plasma deposited layers, which layers comprise one or more first pre-selected functional group species, which functional group species are interactible with a pre-selectable second species.

DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS AND PROCESS UTILIZING SUCH DEVICE

The present invention relates to a device for investigating reactions between interactive chemical and/or biological species, to a process for providing such a device, and to a process for investigating chemical and/or biological interactions, for example biomolecular interactions, utilizing such a device.

Under chemical and/or biological interactions is also understood chemical and/or biological reactions.

Interactions of specific compounds with solid surfaces play a crucial role in chemical and biological phenomena and areas including analysis techniques such as RIA's, ELISA's.

For investigating and sensing surface interactions a 'sensitive' surface is required.

To study real time surface interactions several techniques are available such as ellipsometry, reflectometry and surface plasmon resonance spectroscopy (SPR). These techniques have in common that they use the reflectance of light, generated by a laser, to analyze the growth or desintegration of a layer of for instance biological molecules at a surface.

For these techniques, a reflecting surface is necessary. In the case of SPR, a surface comprising a free electron metal for example gold is most frequently used.

In order to utilize this technique for investigating other interactions, besides the interaction of (bio)molecules with free electron metal surfaces, the free electron surfaces have been modified, for instance, by the adsorption of bio-molecules such as proteins and the coating thereof with polymeric layers in a solvent cast or spin coat procedures.

Methods have also been developed to provide gold surfaces with specific chemical groups for the

immobilization of proteins, which surfaces are subsequently utilized for studying the interactions with other (biological) substances such as antibody-antigen interactions.

Methods for generating SPR sensor surfaces include arranging an organic surface onto a gold layer by means of a wet chemistry procedure such as solvent casting or spin coating before carrying out a plasma etching procedure.

A further method includes adsorption of a chemical functional surfactant, by means of a wet chemistry procedure, on the surface to be modified and the subsequent immobilization of the surfactant by a plasma such as an argon plasma, so called plasma immobilization.

Disadvantages of these known techniques include the lack of stability of the functional surface layers.

An object of the present invention is to provide an improved device for investigating the reactions between interactive chemical species.

According to a first aspect of the present invention there is provided a device according to any of the claims 1 to 5.

The device according to the present invention provides a good attachment of the plasma deposited layer, a good stability thereof and a device exhibiting good sensitivity, whereby the substrate is provided with a functional layer, the functionality of which can be provided by groups such as amine, carboxylic acid, hydroxyl, acid chloride, isocyanate, aldehyde, anhydride, epoxide, and thiol groups for example.

According to a second aspect of the present invention, there is provided a process according to any of the claims 6 to 12 for providing the device according to the present invention.

Since a functional group layer is plasma deposited, control over the deposition thereof can be accurately carried out, whereby very thin layers can be

deposited thus providing very sensitive devices, without the need for firstly arranging an organic layer by wet chemical methods on the substrate before any further investigation can be carried out.

The process according to the present invention provides a good controllability.

In contrast to processes for providing sensor devices, wherein layers are arranged on a substract by wet chemical processes which are often time consuming, difficult to carry out, and often result in undesirably thick layers exhibiting a subsequent lack of sensitivity if a great deal of care in not applied, the process according to the present invention is extremely flexible to work and easy to effect and offers a good cost efficiency.

Plasma deposition procedures involve the deposition of organic species from the plasma phase on a substrate. For instance by applying a (volatile) monomer as the gas phase an organic layer the structure of which resembles the corresponding polymer can be deposited. By applying a (volatile) monomer that possesses a chemical functionality a chemical functional polymeric layer can be obtained.

The plasma may be deposited from a monomer preferably being selected from the group consisting essentially of:

- unsaturated monomers; acrylic acid, allyl amine, allyl isocyanate, allyl mercaptan, methacrylic acid, allyl alcohol, allyl acetate, allyl acetic acid, allyl glycidyl ether, 3 allyloxy, 1-2 propanediol, vinyl acetate, acrylic acid halides,

- saturated monomers; alcohols such as methanol, ethanol propanol, acids such as propionic acid, acetic acid and the like, formaldehyde, propionic aldehyde, glutardialdehyde, aminoethane, aminoethanol, ethylene oxide, acetone methane, ethane, propane and the like, whereby the substrate is provided with the corresponding functionality.

Apart from the plasma deposition of saturated and unsaturated monomers, a functionality can be created in situ, i.e. in the plasma layer, by means of rearrangements of (cyclic) monomers or reaction between a mixture of plasma gases for example, whereafter this in-situ created functionality can be deposited.

Surfaces with a high surface energy, such as metal surfaces in general, may give rise to a rapid surface hydrophobisation due to contamination of the surface by species from its environment. This surface contamination may be disastrous for further surface modification for instance with respect to the stability of the final surface. Therefore this surface contamination should be prevented as much as possible by storing the surfaces in an inert atmosphere and reduction of the time between surface preparation and modification or the surface needs to be cleaned before modification. Plasma etching offers an excellent method for this cleaning. Plasma cleaning is fast and is a clean process in itself since it does not involve the use of organic solvent or substantial amounts of reagents that may have adverse effects on the environment. For the present invention it is advantageous to include an in situ plasma cleaning step of the substrate before the actual modification by plasma deposition.

According to a further aspect of the present invention there is provided a process for investigating the interaction of chemical and/or biological species, for example real time surface interactions, according to claims 14 or 15.

The invention will now be further clarified by way of the following examples, with reference to figure 1 which graphically shows the immobilization of albumins onto a COOH disk as carried out in example 4.

Example 1

Preparation of carboxylic acid functional gold surfaces.

Gold coated glass discs (60) were placed in the central position of the plasma reactor which consisted of a glass tubes (l = 150 cm, ϕ= 10 cm) with three electrodes positioned at the outside of the glass tube with the powered electrode in the center and two grounded electrodes positioned at 30 cm distance on both sides of the powered electrode. The electrodes were connected to an RF-generator (13.56 MHz, ENI ACG-3, ENI Power Systems) through a matching network (ENI Matchwork 5) and a matching network control unit (ENI TH-1000, ENI). The generator was controlled by a timer (Apple Ile computer with a time control program).

The reactor was evacuated to a pressure less than 0.001 mbar by a rotary pump (DUO 004 B, Pfeifer) which was equipped with a filter (ONF 025, Pfeifer) to prevent oil back streaming. The pressure was measured by a pressure gauge (Baratron 628A01MDE, MKS Instruments) and read from a display module (PR4000, MKS Instruments). An air flow of 5 sccm/min resulting in a pressure of about 0.12 mbar, was established for 5 minutes after which the discs were treated with a dynamic air plasma (85 W) for 1 minute at the same flow conditions. Air flow was controlled by a mass flow controller (type 1259 + PR3000 control unit, MKS Instruments). After the plasma treatment the air flow was continued for 2 minutes and then stopped and an acrylic acid flow was established through the reactor via a direct monomer inlet resulting in a pressure of about 0.03 mbar. To prevent the acrylic acid to reach the pump after leaving the reactor, the acrylic acid flow was bypassed through a cold trap that was cooled with liquid nitrogen. The temperature of the acrylic acid in the storage container was room temperature. After two minutes the surfaces were treated with 5 pulses of an acrylic acid plasma at a discharge power of 75 (W), the pulses being separated from each other by 30 seconds of acrylic acid flow through the

reactor. After the final pulse the surface were exposed to 2 additional minutes of acrylic acid flow whereupon the acrylic acid flow was stopped and the reactor was brought to atmospheric pressure with air.

Example 2

Preparation of amine functional surfaces

Gold coated glass discs (60) were placed in the plasma reactor as described in example 1. The reactor was evacuated to a pressure of less than 0.05 mbar and an air flow of 5 sccm/min was established for 5 minutes whereupon the discs were treated with a dynamic air plasma (85 W) for 1 minute at the same flow conditions. Then air flow was stopped and an allyl amine flow (0.07 mbar) was established through the reactor the temperature of the monomer storage container was 36 °C. After two minutes the surfaces were treated with 10 pulses of an allyl amine plasma at a discharge power of 75 W separated from each other by 10 seconds of allyl amine flow through the reactor. After the final pulse the surfaces were exposed to 2 additional minutes of allyl amine flow after which the allyl amine flow was stopped and the reactor was brought to atmospheric pressure with air.

Example 3

Coupling of CMD onto amine functionalized gold surfaces.

Carboxymethyl cellulose (100 mg) was dissolved in 10 ml 0.05 M 2-(N-morpholino) ethanesulfonic acid after which 5 mg N-hydroxysuccinimid was added. After complete dissolution of this reagent 20 mg N-(3-dimethylaminopropyl)-N' ethylcarbodiimide was added. After 3 minutes activation, an amine functionalized gold surface was incubated with 1 ml of this carboxymethyl dextran solution for 2,5 hours. Then the surfaces were rinsed with phosphate buffered saline, and water and vacuum dried. The whole immobilization procedure was performed at room temperature.

Example_4

Immobilization of albumin on a COOH-functionalizes sensing device.

A sensor device, that was COOH-functionalized by the plasma deposition method was used for the immobilization of albumin. During the immobilization procedure that was performed at 22.5°C the surface events were monitored by Surface Plasmon Resonance Spectroscopy of which the results are given in figure 1. After mounting the functionalized sensing device in the SPR apparatus, the sensing surface was incubated with 10 mM HEPES buffer for about 5 minutes. Then the HEPES buffer was exchanged for a EDC (20 mg/ml)-NHS (4 mg/ml) solution in water. After 5 minutes activation the EDC/NHS solution was exchanged for an albumin solution (2 mg/ml in 10 mM HEPES) and an immobilization time of 15 minutes was applied. Then the sensing surface was rinsed with HEPES buffer and the stability of the immobilized albumin in HEPES buffer was monitored for 3 minutes after which the rinsing procedure with HEPES buffer was repeated. To study the stability of the immobilized albumin in 0.1 N HCl the HEPES buffer was replaced by 0.1 HCl and the sensing surface was incubated in this solution for 3 minutes after which 0.1 N HCl was replaced for fresh 0.1 N HCl and the measurement was continued for 3 minutes. Then the surface was rinsed with 0.1 N HEPES buffer again an incubation of the sensing surface was proceeded in this buffer for a final 5 minutes.

The results show that upon activation of the sensing surface with EDC/NHS and subsequent immobilization of albumin and rinsing with HEPES buffer the response increases with about 700 milli-degrees indicating the immobilization of albumin on the COOH-functionalized sensing surface. Rinsing of the surface with 0.1 N HCl only resulted in a decrease of the signal of about 30 milli-degrees, showing that the albumin immobilization is very stable.

CLAIMS

1. Device for investigating reactions between interactive species, said device comprising:

- one or more plasma deposited layers, which layers comprise one or more first pre-selected functional group species, which functional group species are interactible with a pre-selectable second species.

2. Device according to claim 1 wherein the plasma deposited layer is supported on a substrate.

3. Device according to claims 1 or 2 further comprising a film of a free electron metal, preferably selected from the group consisting essentially of copper, silver, aluminum and gold.

4. Device according to claim 3 wherein the plasma deposited layer is arranged directly on the free electron metal film.

5. Device according to any of the previous claims, wherein the plasma deposited layer, comprises one or more chemical and/or biological functional groups.

6. Device according to claim 5 further comprising one or more wet chemically deposited layer(s), arranged on the plasma deposited layer.

7. Process for providing a device according to any of the previous claims, comprising the step of depositing a gas plasma layer onto a pre-selected substrate in order to provide the substrate with a predetermined functionality.

8. Process according to claim 7 wherein the plasma layer is directly deposited onto the substrate and/or onto a metal film arranged on the substrate.

9. Process according to claims 7 or 8 wherein plasma is deposited from a monomer/ oligomer/ polymer in gas form, preferably being a monomer, said monomer being saturated, partially saturated or unsaturated.

10. Process according to any of the claims 7-9 wherein the substrate is subjected to a pre-cleaning step

comprising pre-treating the substrate by means of a plasma etching step before the plasma deposition step said pre-cleaning step preferably comprising pre-treatment with air plasma.

11. Process according to any of the claims 7-10 wherein the gas plasma is deposited under the following conditions:

- a discharge power of upto 5000 W, preferably upto 500 W,
- an exposure duration of upto 1000 s, preferably upto 100 s,
- a plasma gas flow of upto 10000 cm^3/min, preferably upto 100 cm^3/min,
- a pressure of upto 1 bar, preferably from between 0,001-50 mbar,
- a frequency comprising one or more of DC, AC, RF, and the MW, preferably from between 2-60 Mhz.

12. Process according to claim 11 wherein the discharge power is pulsed to the plasma, the pulse discharges being separated by:

- upto 1000 s preferably upto 100 s.

13. Process according to claims 11 or 12 wherein the substrate is treated in an after-glow.

14. Process according to claims 11-13 wherein following pulse discharge, the substrate is after-treated with a pre-selected gas, which gas optionally comprises the one or more functional groups which have been plasma deposited.

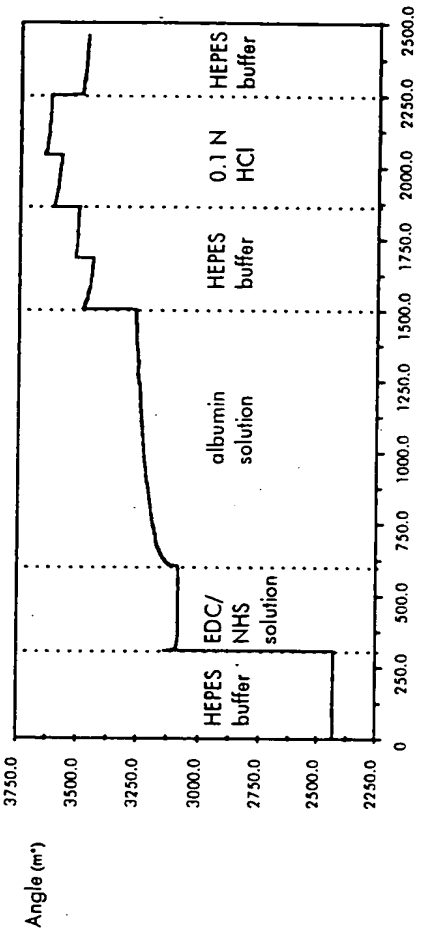
15. Process according to any of the claims 7-14 comprising the further step of wet chemically arranging one or more biological and/or chemical functional layer(s) on the plasma deposited layer.

16. Device according to claims 1 to 6, obtainable according to a process according to any of the claims 7 to 15.

17. Process for investigating the interaction, for example real time surface interaction, of pre-determined chemical and/or biological species, comprising

the steps of analyzing the interaction between the species arranged on a device according to any of the claims 1 to 6 and/or 16.

18. Use of a device according to any of the claims 1-6, and/or 16 for investigating the reaction between chemically interactive species, and especially for use in SPR.


HEPES buffer
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HEPES buffer
albumin solution
EDC/ NHS solution
HEPES buffer
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Figure 1

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